Supplementary Methods:

Patient Selection

After Institutional Review Board (IRB) approval, the Emory University kidney transplant waitlist as of August 10, 2020 was queried for candidates residing in the state of Georgia, receiving hemodialysis or peritoneal dialysis. This data was next integrated with the COVID-19 case rate by county as of August 18, 2020 as provided by the Georgia Department of Public Health (DPH).[S1] 400 waitlist candidates were randomly selected from Georgia counties with a case rate above the average (2229 cumulative cases per 100,000 residents). Demographic data, including age, sex, race, time since referral, and dialysis type, were collected for each patient. Patients with a positive serologic result for SARS-CoV-2 antibodies were contacted by telephone and asked whether they had experienced symptoms of COVID-19, whether they had a prior positive test result, and their date of first vaccination if applicable.

COVID serology testing

For each of the 400 selected patients, two serum samples were screened: the most recent sample collected (through September 2020) and a sample predating the start of the COVID-19 pandemic (prior to December 2019). A Luminex-based assay (LABScreen™ COVID Plus, One Lambda, Inc.) was used to determine serologic status. The assay includes four distinct fragments of SARS-CoV-2 Spike proteins, namely; 1) Full Spike extracellular domain; 2) Spike S1; 3) Spike, Receptor Binding Domain (RBD); and 4) Spike S2. The fifth target is the SARS-CoV-2 Nucleocapsid Protein (NC). Additionally, the kit incorporates Spike S1 fragments from six other coronaviruses, namely HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, MERS-CoV and SARS-CoV.[S2]

Antibody detection on antigen-coated microparticles was performed as follows: Five microliters of viral antigen coated beads were admixed with 20 µl of a 1:10 dilution, in PBS, of patient or control serum that

was pre-treated with 10mM EDTA. The serum/bead admixture was incubated in the dark at 20–25°C for 30 minutes with gentle rotation followed by three sequential washes, each using 150µl wash buffer (WB) (OLI Cat. # LSPWABUF). Following incubation, 25µl of a pre-titered PE-conjugated anti-human IgG (Jackson ImmunoResearch, West Grove, PA. : CAT#: 109-116-170) was added. Next, the beads were vortexed and incubated, in the dark, for 30 minutes at 20 - 25º C with gentle shaking. Finally, the beads were washed twice with 150µl WB. Microparticles were resuspended in 75µl of 1X PBS and analyzed on a Luminex FLEXMAP 3D® instrument (Luminex Corp. Austin, Tx.).

Established cutoffs by trimmed mean fluorescent intensity were used to determine a positive result (Supplementary Table 1). For candidates with a COVID positive serologic result, all available sequential samples were tested to determine the earliest positive date. Additionally, interim 6 month follow-up testing was performed in April 2021 to determine the longevity of the antibody response.

HLA antibody testing

HLA antibody testing was performed for clinical purposes at the time each sample was received. HLA antibody testing was performed using both a screening assay (FlowPRA™, Class I and Class II; One lambda, Inc.) and a single-antigen bead-based specificity assay (LABScreen™ Single Antigen, One lambda, Inc. LS1A04, Lot 10 and LS2A01, Lot 13).

For patients positive for antibodies against SARS-CoV-2, the timing of seroconversion was cross-referenced with HLA antibody testing results.

Statistical Tests

Statistical analyses were performed using R version 4.0.3. Demographic and clinical variables were compared between patients with positive and negative SARS-CoV-2 serology using chi-squared and Wilcoxon-rank-sum testing. A p value of 0.05 was used to determine statistical significance. The

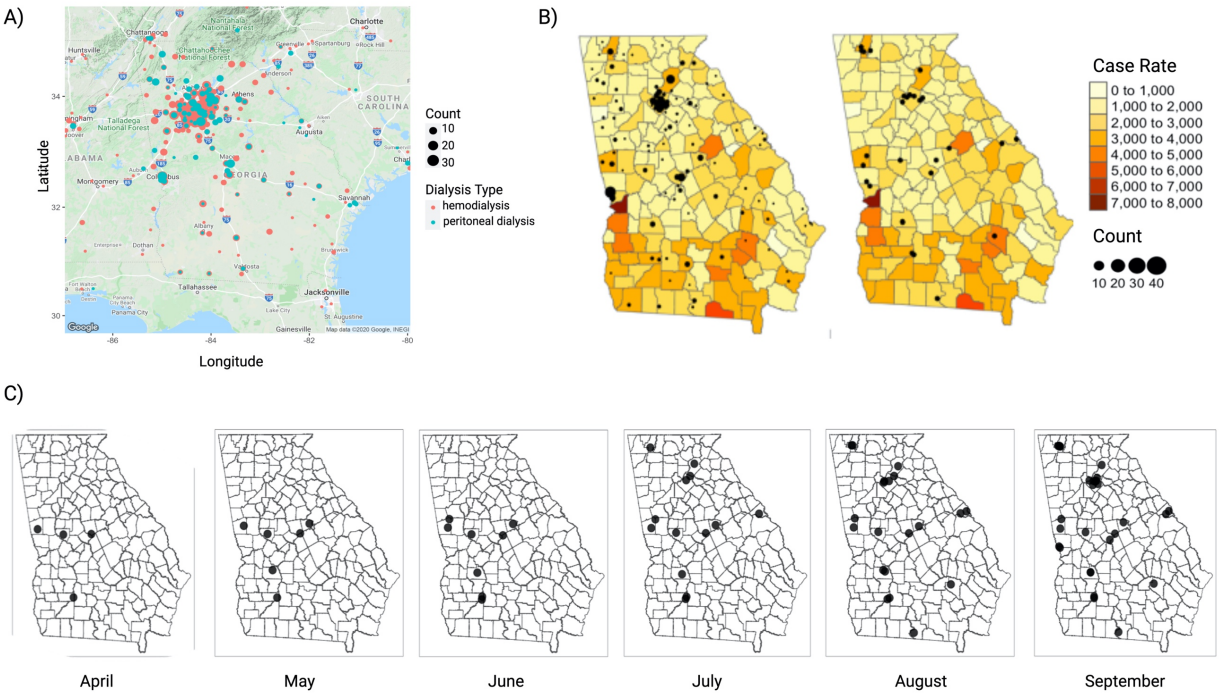
observed case rate for each county was calculated as number of positives divided by total number tested, and these rates were plotted against the case rate published by the Georgia DPH.

Google maps API was applied using the R package 'ggmap' to geocode each patient, using their zip code, with a latitude and longitude using coordinate reference system EPSG:4326.[S3] In order to perform spatial analysis, coordinates were transformed to a planar coordinate reference system with a Robinson projection using the same WGS84 datum. Using the R statistical package 'spatstat,' conditional Monte Carlo testing was performed to assess for clustering of positive cases.[S4]

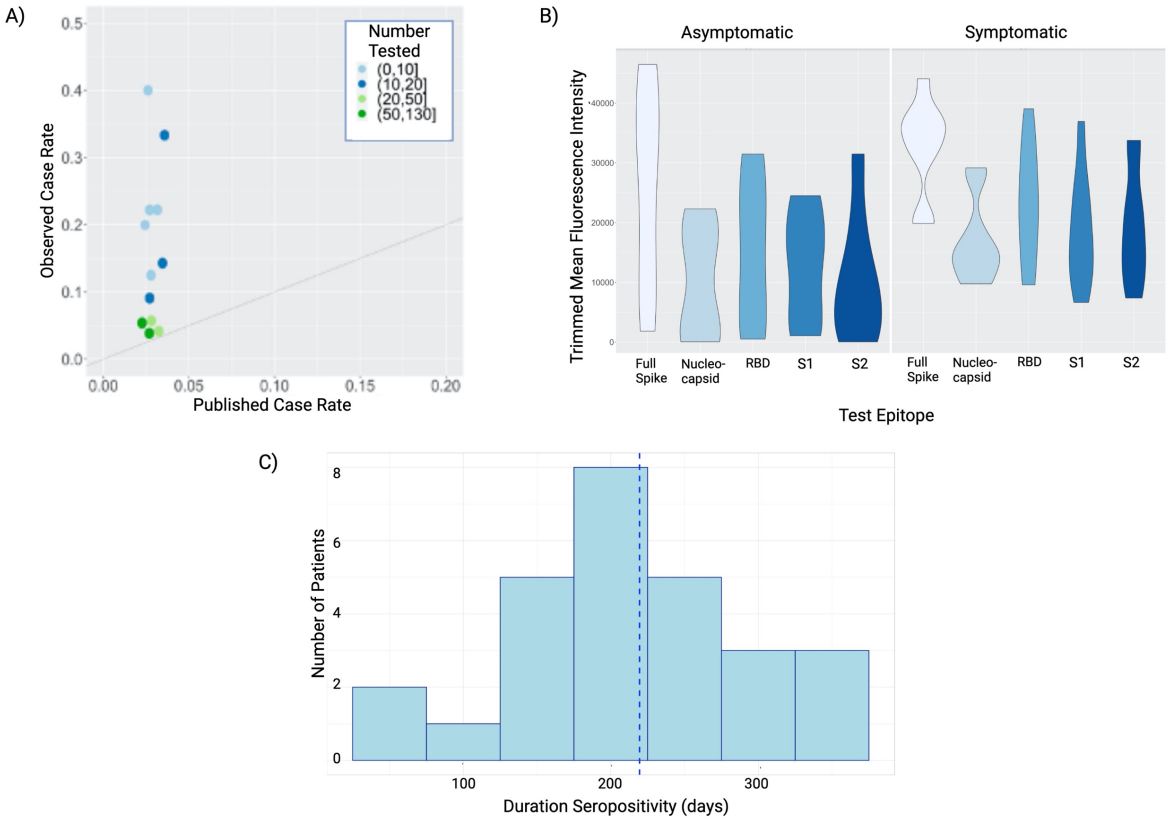
Supplementary Results:

Geographic Analysis

The case rate for each county was calculated as the fraction of candidates who were positive out of all patients tested. For counties with waitlist candidates who tested positive, the case rate published by the Georgia DPH was plotted against the observed case rate (Supplementary Figure 2a). Observed rates were higher than published case rates in all counties with positive cases. However, residuals were inversely related to the number of patients tested. Geocoded patient data was used to plot seroconversions in the order of appearance (Supplementary Figure 1). Conditional Monte Carlo testing using quadrat counts demonstrated no spatial correlation to suggest clustering of positive cases.



Supplementary Figure 1. Geographic analysis of SARS-CoV-2 serology in Georgia kidney transplant waitlist candidates. Figure 1A demonstrates the location and method of dialysis for kidney transplant candidates on the waitlist at Emory University. These patients were filtered down to those only in the state of Georgia. 400 individuals were selected for SARS-CoV-2 antibody testing based on published case rates in their county of residence (Figure 1B, left image). Of the 400 patients tested, 28 were seropositive for antibodies against SARS-CoV-2 (Figure 1B, right image). Sequential maps demonstrating the chronological appearance of antibodies directed against SARS-CoV-2 were generated (Figure 1C). Spatial analysis of this data did not demonstrate any evidence of clustering.



Supplementary Figure 2. Analysis of SARS-CoV-2 Seropositive Patients. For all Georgia counties with seropositive patients, the observed case rate detected in the Emory kidney transplant waitlist population was compared to the published case rate for all individuals in the state of Georgia, provided by the Georgia Department of Public Health (Figure 2A). In all counties with seropositive candidates, the observed case rate was higher than that published for the county. However, residual differences were inversely related to the number of waitlist candidates tested. The MFI for antibodies directed against each epitope was compared for seropositive patients who were symptomatic or asymptomatic (Figure 2B). While symptomatic patients had a higher group average MFI for each epitope, this difference was not statistically significant. Seropositive patients were subject to repeat testing after a 6 month interval, and all patients maintained seropositivity on follow up samples (Figure 2C), with a mean follow up period of 220 days.

PRA Records for SARS-Cov-2 Seropostive Candidates

Patient_ID	Age (years)	Gender	Ethnicity	Etiology of Renal failure	HTN (yes/no)	DM (yes/no)	BMI	Method of dialysis	History of blood transfusions	Previous transplants	Multiparous (yes/no)	SARS-CoV-2 antibody test positive date (mo.yr)	Immunosuppressed at time of infection	cPRA	FlowPRA prior to COVID	FlowPRA after COVID
1	30	M	AA	HTN	yes	no	23	PD	no	no	NA	September	no	10	0, 0	0, 0
2	40	M	AA	UNK	yes	no	28	PD	no	no	NA	Ab positive September, PCR positive 11/2020	prednison 40 q day	34	9, 10	10, 7
3	39	M	AA	HTN	yes	no	23	PD	no	no	NA	September	no	16	0, 0	0, 0
4	30	F	AA	FSGS	yes	no	35	HD	no	no	no	July	no	7	46, 0	0, 0
5	52	M	AA	HTN	yes	yes	31	HD	no	no	NA	August	no	0	0, 0	0, 0
6	52	M	H	DM	yes	yes	24	HD	no	no	NA	July	no	0	0, 0	0, 0
7	65	M	AA	DM	yes	yes	26	PD	no	no	NA	PCR positive 4/14, ab pos May	no	21	0, 11	0, 0
8	44	M	AA	Congenital obstructive uropathy	yes	no	27	HD	no	2	NA	April	no	100	85, 89	95, 90
9	39	M	A	HTN	yes	no	37	HD	no	no	NA	September	no	2	0, 0	0, 0
10	28	F	H	MGN	yes	no	26	PD	no	no	G1P1	July	no	11	0, 14	0, 15
11	22	F	Pacific Islander	UNK	yes	no	37	PD	yes	1	no	July	no	100	84, 71	81, 71
12	68	M	AA	DM	yes	yes	30	HD	no	no	NA	August	no	0	0, 0	0, 0
13	36	M	AA	HTN	yes	no	28	PD	no	no	NA	July	no	3	0, 0	0, 0
14	47	M	H	DM	yes	yes	29	HD	no	no	NA	August	no	0	0, 0	0, 0
15	59	M	C	DM	yes	yes	31	HD	no	no	NA	September	no	0	0, 14	0, 15
16	49	M	AA	HTN	yes	no	29	HD	no	no	NA	April	no	65	0, 11	0, 11
17	72	F	C	HTN	yes	yes	30	HD	no	no	NA	August	no	3	0, 0	8, 0
18	39	M	H	GN	yes	no	33	HD	no	1	NA	July, interesting hospitalized in Nov 2020	prednisone 10 qday	96	16, 88	30, 86
19	41	F	C	HTN	yes	no	25	HD	no	no	no	August	no	61	0, 0	26, 0
20	37	M	AA	DM	yes	yes	21	HD	no	no	NA	August	no	52	37, 0	27, 0
21	53	M	AA	DM	yes	yes	27	HD	no	no	NA	September	no	0	0, 0	0, 0
22	37	M	AA	HTN	yes	no	28	HD	yes	no	NA	April	no	14	0, 04	0, 04
23	56	M	AA	DM	no	yes	35	HD	no	no	NA	May	no	0	0, 0	0, 0
24	51	M	AA	HTN	yes	no	31	HD	no	no	NA	June	no	0	0, 0	0, 0
25	49	M	AA	UNK	yes	no	30	PD	no	no	NA	September	no	15	0, 09	0, 05
26	42	F	AA	DM	yes	yes	32	HD	yes (at least 3)	no	G5P2	August	no	93	73, 0	74, 0
27	43	M	C	FSGS	no	no	26	PD	no	no	NA	September	no	0	0, 0	0, 0
28	33	F	AA	SLE	yes	no	22	HD	yes (up to 6)	1	no	June	hydroxychlorquine	100	99, 99	99. 99

Abbreviations: M (male), F (female), AA (African American), A (Asian), H (Hispanic), C (Caucasian), HTN (hypertension), DM (Diabetes Mellitus), HD (hemodialysis), PD (peritoneal dialysis), Ab (antibody), PCR (polymerase chain reaction)

Supplementary Table 1. Details of Seropositive Patients and Pre/Post Seroconversion FlowPRA Testing. Relevant details for each seropositive patients, including prior sensitizing events and demographic variables, are provided in addition to pre/post exposure FlowPRA results.

WORKSHEET

LABScreen™ COVID Plus, Lot 001

Name

☐ Male
☐ Female

Specificity Assignment

Patient HLA Typing

Donor HLA Typing

Date Collected

Date Tested

Changes from Previous Lot: N/A

Changes from Previous Revision: N/A

	Bead ID	Antigen ID	Established Cut-off Values*		Results
			LABScan™ 100	LABScan3D™	
1	1	NC	NA	NA	
2	2	PC	NA	NA	
3	25	SARS-CoV-2 Spike	7500	7500	
4	38	SARS-CoV-2 Spike S1	4000	4000	
5	50	SARS-CoV-2 Spike RBD	3500	5500	
6	60	SARS-CoV-2 Spike S2	1900	3500	
7	67	SARS-CoV-2 Nucleocapsid Protein	3500	7500	

	Bead ID	Antigen ID	Mean Values from Negative Samples**		Results
			LABScan™ 100	LABScan3D™	
8	68	HCoV-229E Spike S1	3068	5568	
9	72	HCoV-HKU1 Spike S1	2614	4251	
10	80	HCoV-NL63 Spike S1	1043	1927	
11	91	HCoV-OC43 Spike S1	3127	5685	
12	94	MERS-CoV Spike S1	10	17	
13	98	SARS-CoV Spike S1	92	135	

*Baseline calculation applied to trimmed mean value

**200 samples collected in the US prior to December 2019. Included for cross-reactivity information only and does not impact final positive assignment

Antigen Distribution	
Ag	#
SARS-CoV-2 Spike	1
SARS-CoV-2 Spike S1	1
SARS-CoV-2 Spike RBD	1
SARS-CoV-2 Spike S2	1
SARS-CoV-2 Nucleocapsid Protein	1
HCoV-229E Spike S1	1
HCoV-HKU1 Spike S1	1
HCoV-NL63 Spike S1	1
HCoV-OC43 Spike S1	1
MERS-CoV Spike S1	1
SARS-CoV Spike S1	1

Test Performed by Date

Read by Date

Date

Supplementary Data. MFI Cutoffs for SARS-CoV-2 Luminex beads

Supplementary References

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